

Appendix 1

Composition of CAMR MYCOBACTERIA MEDIUM

Stock solutions.	mg l ⁻¹	Stock Solutions	mg l ⁻¹
Amino acids			
L- alanine	100	L-leucine	100
L-arginine	100	L-lysine	100
L-asparagine	2000	L-methionine	100
L-aspartic acid	100	L-phenylalanine	100
L-cysteine	500	L-proline	100
L-glutamine	100	L-serine	100
L-glutamic acid	100	L-threonine	100
L-glycine	100	L-tryptophan	100
L-histidine HCl	100	L-tyrosine	50
L-isoleucine	100	L-valine	100
Inorganic salts			
CaCl ₂ · 2H ₂ O	0.55	NaMO ₄ · 2H ₂ O	1.2
MgSO ₄ · 7H ₂ O	214	NiSO ₄ · 6H ₂ O	0.53
NH ₄ VO ₃	1.2	FeSO ₄ · 7H ₂ O	10
ZnSO ₄ · 7 H ₂ O	28.75	KH ₂ PO ₄	220
CoCl ₂ · 6H ₂ O	0.48	Na ₂ SO ₄	150
CuSO ₄ · 5H ₂ O	0.025	KOH	56
MnCl ₂ · 4H ₂ O	0.02		
Vitamins and co-factors			
inositol	2	nicotinamide	1
thiamine HCl	2	biotin	0.1
calcium -pantothenate	2	DL-thioctic acid	0.1
coenzyme A	0.1		
Other			
ACES buffer	10000	haemin	2.0
NaHCO ₃	42	sodium pyruvate	1000
glutathione (reduced)	500	α-ketoglutarate	1000
glycerol	2 ml	Tween® 80	2.0 ml

Stock solution formulations.

(CAMR MYCOBACTERIA MEDIUM).

Stock solutions.	g l ⁻¹	mg l ⁻¹	Stock Solutions	g l ⁻¹	mg l ⁻¹
<u>Solution 2.</u>			<u>Solution 6.</u>		
CaCl ₂ · 2H ₂ O		55.5	sodium pyruvate	100	
MgSO ₄ · 7H ₂ O	21.4		<u>Solution 7.</u>		
NH ₄ VO ₃		117	α-ketoglutarate	100	
ZnSO ₄ · 7 H ₂ O	2.875		<u>Solution 8.</u>		
<u>Solution 3.</u>			inositol		200
CoCl ₂ · 6H ₂ O		47.6	thiamine HCl		200
CuSO ₄ · 5H ₂ O		2.5	calcium -		
MnCl ₂ · 4H ₂ O		2.0	pantothenate		200
NaMO ₄ · 2H ₂ O		121	nicotinamide		100
NiSO ₄ · 6H ₂ O		52.6	biotin		10
conc. HCl		0.5 ml	<u>Solution 9.</u>		
<u>Solution 4.</u>			DL-thioctic acid	1.0	
FeSO ₄ · 7H ₂ O	1.0		ethanol		950 ml
Conc. HCl		0.5 ml	<u>Solution 10.</u>		
<u>Solution 5.</u>			coenzyme A	1.0	
L- alanine	1.0		<u>Solution 11.</u>		
L-arginine	1.0		haemin	2.0	
L-asparagine	20.0		KOH	56	
L-aspartic acid	1.0				
L-glutamine	1.0				
L-glutamic acid	1.0				
L-glycine	1.0				
L-histidine HCl	1.0				
L-isoleucine	1.0				
L-leucine	1.0				
L-lysine	1.0				
L-methionine	1.0				
L-phenylalanine	1.0				
L-proline	1.0				
L-serine	1.0				
L-threonine	1.0				
L-tryptophan	1.0				
L-valine	1.0				

All solutions were prepared with high quality Millepore water.

Preparation of CAMR MYCOBACTERIA MEDIUM.

Solution	Quantity
ACES buffer	10.0 g
KH ₂ PO ₄	0.22 g
Na ₂ SO ₄	0.15 g
Millepore water	500 ml
Solution 2	10 ml
Solution 3	10 ml
Solution 5	100 ml
Solution 6	10 ml
Solution 7	10 ml
Solution 8	10 ml
Solution 9	0.1 ml
Solution 10	0.1 ml
L-cysteine HCl	0.5 g
Glutathione (reduced)	0.5 g
L-tyrosine	0.05 g
NaHCO ₃	0.042 g
Glycerol	0.2 ml
Solution 4	10 ml
Adjust pH to 6.5 with 20% KOH	
Solution 11	1 ml
Tween® 80	2.0 ml
Millepore water up to 1 litre	

Filter sterilise by passage through 0.07 μ m filter (Sartorius Ltd.)

The above CAMR medium has been refined and non-essential components omitted as below:-

Composition of CAMR Mycobacterium Medium

Chemical	g l ⁻¹	Chemical	mg l ⁻¹
L- alanine	0.1	CaCl ₂ · 2H ₂ O	0.55
L-arginine	0.1	MgSO ₄ · 7H ₂ O	214
L-asparagine	2.0	ZnSO ₄ · 7 H ₂ O	28.75
L-aspartic acid	0.1	CoCl ₂ · 6H ₂ O	0.48
L-glutamic acid	0.1	CuSO ₄ · 5H ₂ O	0.025
L-glycine	0.1	MnCl ₂ · 4H ₂ O	0.02
L-isoleucine	0.1	FeSO ₄ · 7H ₂ O	10
L-leucine	0.1	KH ₂ PO ₄	222
L-serine	0.1	NaHCO ₃	42
L-phenylalanine	0.1		
Sodium pyruvate	1.0	Biotin	0.1
ACES buffer	10	Glycerol	2.0 ml
Tween® 80	2.0		

ACES buffer = N-[Carbamoylmethyl]-2-aminoethanesulfonic acid

Stock solution formulations

Stock solutions.	g l ⁻¹	mg l ⁻¹
<u>Solution 1.</u>		
CaCl ₂ · 2H ₂ O		55.5
MgSO ₄ · 7H ₂ O	21.4	
ZnSO ₄ · 7 H ₂ O	2.875	
<u>Solution 2</u>		
CoCl ₂ · 6H ₂ O		47.6
CuSO ₄ · 5H ₂ O		2.5
MnCl ₂ · 4H ₂ O		2.0
conc. HCl		0.5 ml
<u>Solution 3</u>		
L- alanine	1.0	
L-arginine	1.0	
L-asparagine	20.0	
L-aspartic acid	1.0	
L-glutamic acid	1.0	
L-glycine	1.0	
L-isoleucine	1.0	
L-leucine	1.0	
L-phenylalanine	1.0	
L-serine	1.0	
<u>Solution 4.</u>		
sodium pyruvate	100	
<u>Solution 5.</u>		
FeSO ₄ · 7H ₂ O	1.0	
Conc. HCl		0.5 ml
<u>Solution 6</u>		
Biotin		10

All solutions were prepared with high quality Millipore water.

Preparation of CAMR MYCOBACTERIA MEDIUM.

Solution	Quantity
ACES buffer	10.0 g
KH ₂ PO ₄	0.22 g
Millipore water	500 ml
Solution 1	10 ml
Solution 2	10 ml
Solution 3	100 ml
Solution 4	10 ml
Solution 6	10 ml
NaHCO ₃	0.042 g
Glycerol	2 ml
Solution 5	10 ml

Adjust pH to 6.5 with 20% KOH

Tween® 80 2.0 ml

Millipore water up to 1 litre

Filter sterilise by passage through 0.07 µm filter (Sartorius Ltd.)